PATENT

IN THE UNITED STATES PATENT
AND TRADEMARK OFFICE

Applicants:	Zhang, et al.	I hereby certify that this paper is being deposited with the United States Postal Service on **December 27, 2001**, in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. ET126944685US.
Serial No.:	To Be Determined	
Filed:	Herewith	
For:	An Improved Method for the Production and Purification of Adenoviral Vectors	
Continuation of:	09/556,570	
Filed:	April 24, 2000	
Art Unit:	Unknown	Richard Zimmermann
Examiner:	Unknown	

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant respectfully requests consideration of the following new claims. No fees beyond those enclosed are believed to be due. Should the Commissioner determine otherwise, he is authorized to charge Applicant's deposit account number 13-2855.

IN THE TITLE

Please amend the title as follows:

"Methods for Producing Purified Adenoviral Vectors"

IN THE SPECIFICATION

Please amend the specification by deleting the text at lines 2 - 5 and replacing it with

the following:

The present application is a continuation of U.S. application serial number 09/556,570, filed April 24, 2000, which is a continuation of U.S. application serial number 08/975,519, filed November 20, 1997, which claims benefit of U.S. provisional application serial number 60/031,329, filed November 20, 1996. The entire text of the above-referenced disclosures are specifically incorporated by reference herein without disclaimer.

IN THE CLAIMS

Cancel claims 1-69. Add new claims 70-128 as follows:

70. A method for making a purified adenovirus composition for therapeutic use comprising:

a) growing host cells in a media;

b) providing nutrients to said host cells by perfusion, fed batch or automated roller bottles;

c) infecting said host cells with an adenovirus;

d) lysing said host cells to provide a cell lysate comprising adenovirus; and

e) purifying adenovirus from said lysate by a process other than the use of cesium chloride density gradient centrifugation to provide a purified adenovirus composition.

71. The method of claim 70, wherein the purified adenovirus composition for therapeutic use comprises 70% +/- 10% of the starting PFU.

72. The method of claim 70, wherein the purified adenovirus composition for therapeutic use comprises a substantially purified adenovirus composition.

73. The method of claim 70, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.8 ng/ml.

74. The method of claim 70, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.2 ng/ml.

75. The method of claim 70, wherein the purified adenovirus composition for therapeutic use has an A_{260}/A_{280} ratio of between about 1.2 and 1.3.

76. The method of claim 70, wherein the purified adenovirus composition for therapeutic use has an A_{260}/A_{280} ratio of 1.27 +/- 0.03.

77. The method of claim 70, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.8 ng/ml and an A_{260}/A_{280} ratio of between about 1.2 and 1.3.

78. The method of claim 70, wherein the purified adenovirus composition for therapeutic use has a BSA content below the detection level of a western blot assay.

79. The method of claim 70, wherein the media is serum-free.

80. The method of claim 70, wherein the host cells are grown in a bioreactor.

81. The method of claim 70, wherein the host cells are grown on microcarriers.

82. The method of claim 70, wherein said purified adenovirus composition for therapeutic use comprises an adenoviral vector encoding an exogenous gene construct.

83. The method of claim 82, wherein said exogenous gene construct is operatively linked to a promoter.

84. The method of claim 83, wherein said promoter is SV40 IE, RSV LTR, β-actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

85. The method of claim 82, wherein said exogenous gene construct encodes a therapeutic gene.

86. The method of claim 85, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*, antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-1, MEN II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, GM-CSF, G-CSF, thymidine kinase or p53.

87. The method of claim 86, wherein said therapeutic gene encodes p53.

88. The method of claim 70, wherein said purified adenovirus composition for therapeutic use is a replication-incompetent adenovirus.

89. The method of claim 88, wherein said purified adenovirus composition for therapeutic use is lacking at least a portion of the E1-region.

90. The method of claim 89, wherein the purified adenovirus composition for therapeutic use is lacking at least a portion of the E1A and/or E1B region.

91. The method of claim 70, wherein said host cells are capable of complementing replication.

92. The method of claim 70, wherein said host cells are 293 cells.

93. The method of claim 70, wherein said lysate is treated with a nuclease.

94. The method of claim 70, wherein said purified adenovirus composition for therapeutic use comprises a pharmaceutically acceptable buffer.

95. The method of claim 94, wherein said purified adenovirus composition for therapeutic use provides a unit dose of between 10^3 and 10^{15} PFU/dose.

96. The method of claim 94, wherein the purified adenovirus composition for therapeutic use provides a unit dose of between 10^{10} and 10^{14} PFU/dose.

97. The method of claim 70, wherein said purifying is characterized by a process that includes at least one chromatography step capable of providing a purified adenovirus composition for therapeutic use.

98. The method of claim 70, wherein said purifying is characterized by a process that includes a single chromatography step capable of providing a purified adenovirus composition for therapeutic use.

99. The method for making a purified adenovirus composition for therapeutic use comprising:

a) growing host cells in a media comprising glucose;

b) infecting said host cells with an adenovirus;

c) lysing said host cells by a lysis method other than freeze-thaw to produce a lysate comprising said adenovirus composition; and

d) purifying adenovirus from said lysate to provide a purified adenovirus composition for therapeutic use.

100. The method of claim 99, wherein the purified adenovirus composition for therapeutic use comprises 70% +/- 10% of the starting PFU.

101. The method of claim 99, wherein the purified adenovirus composition for therapeutic use comprises a substantially purified therapeutic adenovirus composition.

102. The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.8 ng/ml.

103. The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.2 ng/ml.

104. The method of claim 99, wherein the purified adenovirus composition for therapeutic use has an A_{260}/A_{280} ratio of between about 1.2 and 1.3.

105. The method of claim 99, wherein the purified adenovirus composition for therapeutic use has an A_{260}/A_{280} ratio of 1.27 +/- 0.03.

106. The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.8 ng/ml and an A_{260}/A_{280} ratio of between about 1.2 and 1.3.

107. The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a BSA content below the detection level of a western blot assay.

108. The method of claim 99, wherein the media is serum-free.

109. The method of claim 99, wherein the host cells are grown in a bioreactor.

110. The method of claim 99, wherein the host cells are grown on microcarriers.

111. The method of claim 99, wherein said purified adenovirus composition for therapeutic use comprises an adenoviral vector encoding an exogenous gene construct.

112. The method of claim 111, wherein said exogenous gene construct is operatively linked to a promoter.

113. The method of claim 112, wherein said promoter is SV40 IE, RSV LTR, β-actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

114. The method of claim 111, wherein said exogenous gene construct encodes a therapeutic gene.

115. The method of claim 114, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*, antisense *abl*, Rb, CFTR, p16, p21,

p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-1, MEN II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, GM-CSF, G-CSF, thymidine kinase or p53.

116. The method of claim 115, wherein said therapeutic gene encodes p53.

117. The method of claim 99, wherein said purified adenovirus composition for therapeutic use is a replication-incompetent adenovirus.

118. The method of claim 117, wherein said purified adenovirus composition for therapeutic use is lacking at least a portion of the E1-region.

119. The method of claim 118, wherein the purified adenovirus composition for therapeutic use is lacking at least a portion of the E1A and/or E1B region.

120. The method of claim 99, wherein said host cells are capable of complementing replication.

121. The method of claim 99, wherein said host cells are 293 cells.

122. The method of claim 99, wherein said lysate is treated with a nuclease.

123. The method of claim 99, wherein said purified adenovirus composition for therapeutic use comprises a pharmaceutically acceptable buffer.

124. The method of claim 123, wherein said purified adenovirus composition for therapeutic use provides a unit dose of between 10^3 and 10^{15} PFU/dose.

125. The method of claim 123, wherein the purified adenovirus composition for therapeutic use provides a unit dose of between 10^{10} and 10^{14} PFU/dose.

126. The method of claim 99, wherein said purifying is characterized by a process that includes at least one chromatography step capable of providing a purified therapeutic adenovirus composition.

127. The method of claim 99, wherein said purifying is characterized by a process that includes a single chromatography step capable of providing a purified therapeutic adenovirus composition.

128. The method of claim 99, wherein the lysis method other than freeze-thaw is a process that includes hypotonic solution, hypertonic solution, impinging jet, microfluidization, solid shear, detergent, liquid shear, high pressure extrusion, autolysis or sonication to produce a crude lysate composition comprising adenovirus.

REMARKS

New claims 70-128 have been added and are considered pending. The Examiner is respectfully requested to consider these claims. Support for claims 70, 99, and126-128 is found throughout the specification, particularly at the following locations: page 11, line 12 through page 109, line 20. Support for claims 71 and 100 is found throughout the specification, particularly at the following locations: page 12, line 5 and page 99, line 28 through page 100, line 8. Support for claims 72 and 101 is found throughout the specification, particularly at the following locations: page 64 lines 13-18. Support for claims 73, 74, 102 and 103 is found throughout the specification, particularly at the following locations: page 92, lines 10-20 and Table 10. Support for claims 75-77 and 104-106 is found throughout the specification, particularly at the following locations: page 91, lines 1-13. Support for claims 78 and 107 is found throughout the original specification, particularly at the following locations: pages 38-41, 43-57, 87, 92, and 93. Support for claims 79 and 108 is found throughout the specification, particularly at the following locations: page 15, lines 16-28, page 16, lines 17-29, page 27, lines 25-30, page 28, lines 1-30, page 100, lines 18 through page 105, line 28 and Tables 12 and 13. Support for claims 80 and 109 is found throughout the specification, particularly at the following locations: page 17, line 24 through page 18, line 18. Support for claims 81 and 110 is found throughout the specification, particularly at the following locations: page 22, line 8 through page 23, line 11. Support for claims 82-96 and 111-125 is found throughout the original specification, particularly at the following locations: page 72, line 11 through page 75, line 18.

It is submitted that each of claims 70-128 should now be indicated to be allowable. Should the Examiner have any questions of form or substance, he or she is invited to contact the undersigned attorney at the number listed below.

Respectfully submitted,

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December 27, 2001